Antagonistic and Plant Growth Promoting Potentials of Indigenous Endophytic Bacteria of Shallots

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Abstract: This research was further study of screening endophytic bacteria that isolated from the roots of healthy shallots. The aim of research was to determine the ability of endophytic indigenous bacteria as anti-microbial agents and plant growth promoters. In this study, there are two experiments: 1) *in vitro* anti-microbial test against pathogens from the group of fungi and bacteria, 2) testing the physiological and biochemical characteristics of endophytic bacteria as potential biocontrol agents and growth promoters, namely production of salicylic acid, siderophore, IAA, lipase and protease enzyme as well as the ability to phosphate solubilization. Endophytic bacteria was capable to inhibiting of pathogenic fungi, except *Bacillus* sp HI that could not inhibit *Foc*. *B. cereus* P14, *B.cereus* Se07, *Bacillus* sp SJI and *S.marcescens* had the ability to inhibit of all three pathogenic fungi (*C. capsici*, *C. gloeosporiodes* and *Foc*). All endophytic bacteria were able to inhibit *Xaa*, but were not able to inhibit *R. solanacearum*. Physiological characteristics of potential endophytic bacteria as biocontrol agents and growth promotor ware the production of salicylic acid, siderophot, IAA, lipase and protease enzymes and phosphate solubilization.

Keywords: Antagonists, Endophytic, salicylic acid, siderophore.

1. Introduction

Bacterial endophytes are bacteria that colonizers of the inner plant tissues where they do not normally cause any substantial morphological changes and disease symptoms [10]. Endophytic bacteria can be isolated from the roots, stems, leaves, flowers, and cotyledons. The bacteria can enter through the seed germination process, the stomata of secondary roots, or through the leaves damage. Endophytic bacteria can be localized to the part where the bacteria begin to enter or spread to other parts of the plant. In plant tissues, bacteria are located between cells or in vascular tissues [20].

Endophytic bacteria can play important roles as biocontrol agents, suppress pathogens, several types of nematodes and insects through direct or indirect mechanisms. The direct mechanism is to produce antimicrobial compounds [18], siderophor and lytic enzymes [8], competitions in iron, nutrition and space, and parasitism. The mechanism indirectly through the induction of systemic resistance to host plants. Induction of systemic resistance (Induced Systemic Resistance = ISR) is the interaction of specific bacteria with roots that allow these plants to develop resistance to potential pathogens [1]. Resistance induction can increase the activity of mechanical endurance genes or host plant metabolites, increasing the strength of cell walls. Physiological changes of host plants through synthesis of phenolic compounds, PR proteins, chitinase enzymes, peroxidases, phenyl alanine liases, polyphenols oxidase, salicylic acid, jasmonic acid and fitoalexin [3]. Endophytic bacteria as a plant growth promote is as a biofertilizer, rhizoremediator, phytostimulator and protect plants from abiotic stress (Induced Systemic Tolerance). Endophytic bacteria provide nutrients for host plants with nitrogen fixation and phosphate solubilition [8], provides Fe through siderophor, and produces phytohormones such as IAA, gibberellins and cytokines.

Important role of endophytic bacteria as biocontrol of plant diseases has been widely reported such as Berg and Hallmann (2006) and Kloepper et al. (2004), reported that endophytic bacteria can reduce disease severity, induce plant defense mechanisms [1], and produces anti-herbivory compounds. Direct biocontrol mechanisms include antibiosis, competition for nutrients and niches, and indirectly by inducing plant resistance.

Screening results of 82 endophytic indigenous isolates from shallots as a biocontrol of Bacterial Leaf Blight (BLB) Disease which has been done before obtained 6 endophytic bacteria that have the potential to be developed as biocontrol agents and biofertiliser for plants. The six endophytic bacteria can suppress the percentage of BLB disease severity on shallots between 17.28 -

64.30% compared to control. Improve the resistance of shallots plants from rather susceptible to resistant. Four endophytic bacteria (*B.cereus* P14, *Bacillus* sp. SJI, *Bacillus* sp. HI and *S, marcescens*) were able to suppress the severity of BLB disease between 58.96 - 64.30% compared to controls. Two endophytic bacteria (*S. marcescens* and *B. cereus* Se07) were only able to reduce the severity of the disease 17.28% and 28.32%, but increase the yield of 212.78% and 214.85% compared to control. When calculated per hectare these two bacteria can increase the weight of bulbs up to 15.12 ton / ha and 15.22 ton / ha, nearing the optimal productivity of shallots 16 ton / ha [15].

In this study, endophytic bacteria were tested as anti-microbial against fungal and bacterial plant pathogen, biochemical and physiological characterization of that bacteria. The discovery of endophytic bacteria suitable for control of plant pathogen and effective method application is a significant contribution for development of science in the field of biological control, and the development of biopesticide production in Indonesia. The objective of the research was to determine the ability of endophytic indigenous bacteria as anti-microbial agents and plant growth promoters.

2. Material & Method

2.1. Endophytic bacteria isolates

Endophytic bacterial isolates used in the study were isolated from healthy roots of shallots in endemic areas of bacterial leaf blight (BLB) diseases. Bakteria was isolated from rooting of healthy shallots from two regencies in West Sumatera namely Solok Regency and Agam Regency. Endophytic bacterial isolates used were SN1E4 (*Bacillus* sp H1), SN2E2 (*Bacillus cereus* Se07), PU2E2 (*Bacillus* sp SJ1), BD4.2E1 (*Bacillus cereus* P14), and ULG1E2 (*Serratia marcescens*).

2.2. In Vitro Assay of Antagonistic Activity against plant pathogenic bacteria

Endophytic bacteria cultured in Nutrient Broth (NB) medium incubated in shaker for 2 x 24 hours, 200 rpm at room temperature. The bacterial culture was centrifuged at 10,000 g for 10 minutes. The supernatant was separated from the pellet. The 0.5 cm diameter sterile filter paper was immersed in the supernatant for 5 minutes, then air dried. The filter paper was prepared on Potato Dextrosa Agar (PDA) medium which has been inoculated with plant pathogenic bacteria *Xanthomonas axonopodis* pv.*allii* (Xaa) and *Ralsonia solanacearum* (*R. solanacearum*) and incubated for 2 x 24 hours [12]. The ability to produced antibiotics was characterized by inhibition zone the paper disc.

2.3. In Vitro Assay of Antagonistic Activity against plant pathogenic fungi

In this assay we used a dual culture method against three major pathogens: *Fusarium oxysporum* f.sp *cubence, Colletotrichum capsici,* and *Colletotrichum gloeosporiodes*, this pathogenic fungi was as a collection of Phytopathology Laboratory Andalas University.

The assay for antagonism was performed on potato dextrose agar (PDA) adopting dual culture method as suggested by Zivkovic et al. (2010). A 5 mm of mycelia agar disc from test fungal pathogen cultures was placed on the one side of a Petri plate containing PDA medium. The plates were then incubated at 25^0C for 24 hrs. A loopful of test antagonistic bacterial culture was then streaked 3 cm away from the disc of pathogen on the same dish. PDA plates inoculated only with pathogens were maintained as control. The zone of inhibition was recorded as the distance between the fungal pathogen and the area of antagonist growth after 7 days.

The percent of inhibition was calculated using the formula:

$$D = \frac{A-B}{A} \times 100\ \%$$

Where D = Percentage of inhibition,
A = Radial of control fungi
B = Radial of treatment fungi.

2.4. Physiological and biochemical character of endophytic bacteria

The bacteria were cultured in Nutrient Agar (NA) medium and purified on the same medium. The purified culture was used for subsequent testing.

2.5. Siderophores Production

Siderophore production was determined through the methods described by Macagnan *et al.* (2008). Endophytic bacteria were grown on King's B (KB) broth medium for 10 days [9]. The culture was centrifuged at 10,000 rpm for 10 minutes. One milliliter of the supernatant was added with 1 ml of Chromo-azurol S, according to the method of Schwyn and Neylands (1987), and then mixed. Siderophore production was indicated by a change in color of the mixture from bluish-red to brown in 15 minutes. Medium containing 2 μmol L-1 Fe^{3+} from a sterile solution of $FeSO_4 \cdot 7H_2O$ was used as control. All treatments were repeated three times.

2.6. Salicylic Acid Production

Endophytic bacterial isolates were cultured in a Nutrient Broth (NB) medium and incubated in shaker at 210 rpm for 48 h at room temperature. Endophytic cultures was centrifuged at 7000 g for 10 minutes. The supernatant was analyzed by capillary electrophoresis using pure salicylic acid as the standard. Capillary electrophoresis was applied at 10 KV, wavelength used 210 nm, and temperature 20^{o}C [16].

2.7. IAA Production

The bacterial isolates were inoculated into 20 mL of Kings B broth supplemented with 0.2 % (v/v) of L-tryptophan and incubated for 10 days at 28^{o}C. After incubation, the culture was centrifuged at 3,000 rpm for 20 min and the supernatant was used for analysing indole 3 acetic acid production [14]. Initially one mL supernatant was mixed with 2 mL of Salkowski reagent (1 ml $FeCl_3$ in 49 ml of perchloric acid 35%) and tubes were incubated in dark for 30 min. The development of the red color was observed as the indication for positive result. Uninoculated growth medium was used as negative control. The supernatant was determined by spectrofotometri with the wavelength of 530 nm.

2.8. Phosphate Solubilization

The endophytic bacterial isolates were screened for phosphate solubilization using the procedure described by Jasim *et al.* (2013). For this, Pikovskaya medium (g/L—glucose 10, tri-calcium phosphate 5, ammonium sulphate 0.5, sodium chloride 0.2, magnesium sulphate heptahydrate 0.1,potassium chloride 0.2, ferrous sulfate heptahydrate 0.002, yeast extract 0.5, manganese (II) sulfate dehydrate 0.002, agar 20, pH 7.0) was used. The media inoculated with the isolates were incubated for 48 h and was observed for the formation clear zone around the colony due to the utilization of tricalcium phosphate present in the medium.

2.9. Protease Activity

Proteolytic activity of the cultures was studied in a medium containing skimmed milk. Zone of precipitation of paracasein around the colonies in the next 48 hours were taken as evidence of Proteolytic activity.

2.10. Lipase Activity

The lipase activity of the endophytic bacterial was determined by supplementing the Nutrient Agar media with 0.01% $CaCl_2.H_2O$, followed by adding sterilized Tween 80 to the media to give a final concentration of 1%. The media was poured into the Petri plates, and presence of opaque halo zone around the colonies was considered as positive [17].

3. Result and Discussion

3.1. *In Vitro* Assay of Antagonistic Activity

The endophytic bacteria were able to inhibit Xaa bacteria, except *B. cereus P14*. All endophytic bacteria used were not able to inhibit *R. solanacearum* (Table 1). Endophytic bacteria are able to inhibition the growth of three plant pathogenic fungi (*Fusarium oxysporum* f.sp *cubence* = Foc, *C capsici* and *C gloeosporiodes*). Only *Bacillus* sp. HI was not able to inhibit the growth of Foc, because the percentage of inhibitory was negative (- 1.19%).

Table 1. Endophytic bacteria ability to inhibit fungi and bacteria pathogen

No.	Endophytic bacteria	% inhibition			Inhibition zone (mm)	
		C.capsici	*C.gleosporaides*	*Foc*	*R. solanacearum*	*Xaa*
1	*B.cereus P14*	28.48	19.79	14.54	0.00	0.00
2	*B.cereus Se07*	18.28	28.06	17.60	0.00	16.25
3	*Bacillus sp SJI*	26.46	10.15	12.76	0.00	18.25
4	*Bacillus sp HI*	2.53	17.08	-1.19	0.00	20.25
5	*S. marcescens*	8.33	28.00	10.98	0.00	17.25

B.cereus P14 was have the highest ability to inhibition growth *C. capsici* with a 28.48%. *B.cereus* Se07 and *S.marcescens* were have the ability to inhibit *C. gloeosporiodes* with 28.06 and 28.00%. *B. cereus* Se07 was have the highest ability to inhibit Foc with 17.60%. While *Bacillus* sp HI was able to inhibit Xaa with the largest inhibition zone is 20.25 mm.

The ability of endophytic bacteria to inhibit *C. capsici* was higher than other pathogenic fungi (*Fusarium oxysporum* f.sp *cubence* and *C. gloeosporiodes*), while the lowest ability was to inhibit Foc,. *Bacillus* sp. HI was not able to inhibition Foc. endophytic bacteria inhibit The growth of *C. capsici* and *C. gloeosporiodes* directly, by inhibiting the growth of the pathogenic fungal hyphae, whereas in Foc, endophytic bacteria lead to depletion of fungal hyphae growth compared with controls.

3.2. Production of Salicylic Acid, Siderofor, IAA and Phosphate Solubilization

The production of salicylic acid, siderophores, IAA and P solubilization was shown in Table 2 and Figure 1. All endophytic bacteria of the characterized *Bacillus* genus are capable of producing salicylate acid and IAA, with varying concentrations. The production of siderophores was only in *Bacillus* sp HI, whereas those were capable of dissolving P was *B. cereus* P14. *S.marcescen* was not produce salicylic acid, but produces IAA, siderophores and was also capable of dissolving P. *B. cereus* P14 was an endophytic bacterium capable of producing the highest salicylic acid and IAA compared to other endophytic bqkteri, with concentration of 14.72 ppm / ml and IAA concentration of 93.16 ppm. While *S.marceacens* was highest capable of diluting P with index value 4.

Table 2. Endophytic bacteria ability to production of salicylic acid, siderophores, IAA and phosphate solubilization

No	Endophytic bacteria	Salicylic acid		IAA		Pelarut P		Siderofor
		Results	Concentration	Results	Concentration	Results	Index	
1	*B.cereus P14*	+	14.72	+	93.16	+	2	-
2	*B.cereus Se07*	+	13.96	+	45.56	-	0	-
3	*Bacillus sp SJI*	+	14.67	+	64.16	-	0	-
4	*Bacillus sp HI*	+	14.4	+	42.56	-	0	+
5	*S. marcescens*	-	0	+	37.96	+	4	+

Note: + = able to produce, - = unable to produce

Production of siderophores was expressed by changes in supernatant color of endophytic bacteria (*Bacillus* sp. HI and *S.marcescens*) In CAS solution to became brown (Figure 1A). The ability to dissolve P was indicated by the presence of a clear zone in bacterial colonies of *B.cereus* P14 and *S.marcescens* on Pikovskaya medium (Figure 1B). All endophytic bacteria tested were able to produce IAA as indicated by the color change of the bacterial supernatant in reagent sowlkesky to became red (Figure 1C).

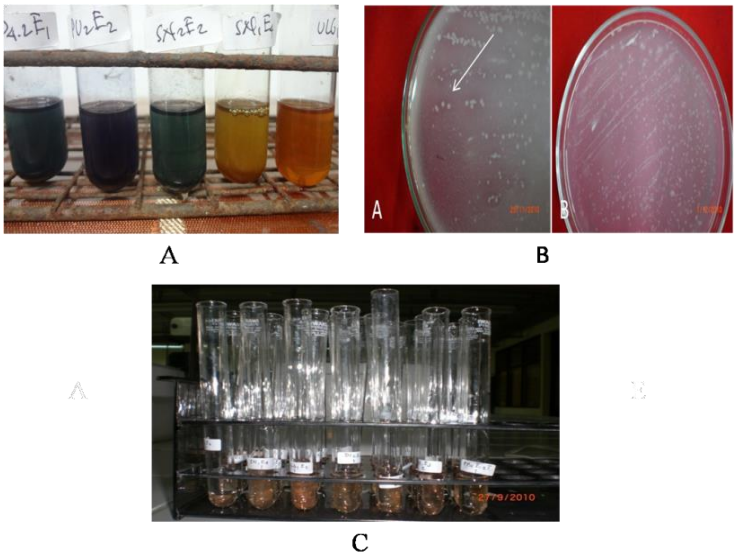


Figure 1. Production of siderophores, IAA and Phosphate Solubilization endophytic bacterial; A. Production of siderophores, B., Phosphate Solubilization C. Production of IAA

3.3. Production of Lipase and Protease Enzymes

The production of protease and lipase enzymes of endophytic bacteria was shown in Table 3 and Figure 2. All endophytic bacteria were capable of producing protease and lipase enzymes. This ability was expressed by clear zones and casein precipitation on skimmed milk agar for the production of protease enzymes (Figure 2A), and clear zones in NA enriched medium for the production of lipase enzymes (Figure 2B).

Table 3. Endophytic bacteria ability to produce Lipase and enzyme Protease enzymes

No	Endophytic bacteria	Lipase enzymes	Protease Enzymes
1	*B.cereus P14*	+	+
2	*B.cereus Se07*	+	+
3	*Bacillus sp SJI*	+	+
4	*Bacillus sp HI*	+	+
5	*S. marcescens*	+	+

Note: : + = able to produce, - = unable to produce

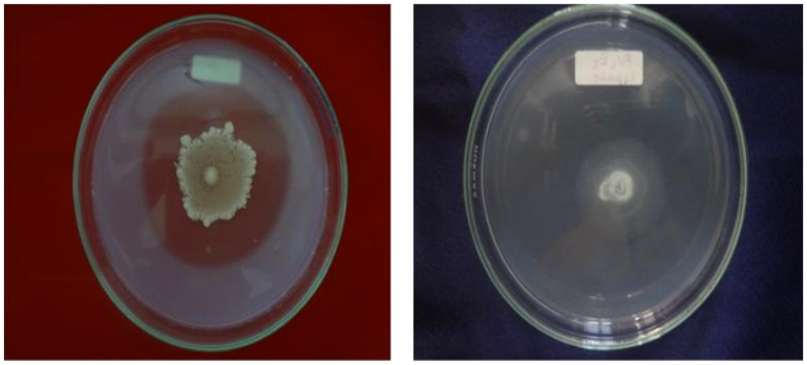
3.4. Discussion

Endophytic bacteria that have been isolated from the roots of healthy shallot plants are 82 isolates. All isolates were selected for their ability as biocontrol of BLB disease and obtained the best 5 isolates, four isolates were from *Bacillus*, and one solate from *Serratia*. Bacteria of the genus *Bacillus* ware more dominant than those of other genera. As reported by Pereira *et al.* (2011), which isolates endophytic bacteria from roots of maize, and acquires more *Bacillus* in association with roots of maize, compared with other genera [13].

In this study, endophytic bacteria as a potential biocontrol agent was characterized their ability to produce salicylic acid, siderophores, IAA, Phosphate solubilization and to produce enzymes. *Bacillus* sp HI had the most complete physiological characteristics compared to other endophytic bacteria, which was the ability to produce salicylic acid, siderophores, IAA, lipase and protease, but not capable of dissolving P. *Bcereus* P14 was able to produce salicylic acid, IAA, lipase protease, and dissolve P, but did not produce siderophores. *B.cereus* Se07 and *Bacillus* SJI were able to produce salicylic acid, IAA, lipase and protease. *S.marcescens* produces IAA, siderophores, dissolves P, lipase and protease enzymes. Kumar *et al* (2016) isolated endophytic bacteria from

Curcuma longa.L and obtained 14 isolates consisting of *B. cereus, B. thuringiensis, Bacillus* sp, *B.putida* and *P.fluorecens* [7]. All of these bacteria produce IAA and dissolve P, only *Bacillus* sp and *P.fluorescens* that produce siderophores.

Although *Bacillus* sp HI had the most complete character, but these endophytic bacteria were not able to inhibit *Foc* and *R. solanacearum*. Its ability to inhibit *C. capsici* and *C. gleosporoid*es was lower than other endophytic bacteria with a percentage 2.53 and 17.08%. So it can be assumed that the endophytic bacteria characteristic was not related to its ability as a pathogenic fungal inhibitor agent, since the inhibition of the fungus is direct while the character possesses was associated with indirect (induced systemic resistance). Hardam *et al* (2015) reported that siderophores produced by endophytic bacteria play an important role in inducing systemic resistance (induced systemic resistance) [4]. The role of endophytic bacteria as a beneficial microbe both as a biocontrol agent for pathogenic microbial and as growth promote was strongly influenced by its interactions with host plants.



A B

Figure 2. The ability of endophytic bacteria to produce protease and lipase enzymes:
A. Production of protease enzymes, B. Production of Lipase enzymes

B.cereus P14, which had complete character, except the production of siderophores, was capable to inhibiting pathogenic fungi (*C. capsici, C. gloeosporiodes* and *Foc*) with the percentages of inhibition 28.48 %, 19.79 %, and 14.54%. In this endophytic bacteria there was a relationship between their characters with its ability as a biocontrol agent. Melnick *et al* (2008) reported that *B. cereus* isolate BT8 from tomato, isolate BP24 from potatoes and formulated with polysilicon surfactant Silwet L-77 (0.24% vol / vol), could inhibit leaf spot disease on cacao caused by *P.capsici* [11]. In the case of BLB disease on shallots, endophytic bacteria also had the highest ability to induce plant resistance to that diseases [15].

B. cereus Se07 was able to inhibit all three pathogenic fungi with the highest inhibition on *C. gloeosporiodes* and *Foc* with the inhibitory percentage was 28.06 % and 17.60%. This bacteria had a character that capable of producing salicylic acid and IAA, which was be related to its ability as a biocontrol agent and plant growth promoter. *S.marcescens* was capable of inhibiting pathogenic fungi *C. capsici, C.* g*loeosporiodes* and *Foc* with percentages of inhibition 8.33%, 28.00%, and 10.98%.. Xu *et al* (2007) reported that *S.marcescens* isolated from *Vaccinium uliginosum* was able to inhibit fungal pathogens in blueberries with the percentage of inhibition 22.7 % -70.5% [19]. Furthermore, according to Resti *et al* (2013), endophytic bacteria *B.cereus* Se07 was able to make shallots tolerant to BLB disease and increase the yield of shallots bulbs [15].

4. Conclusion

Endophytic bacteria was capable to inhibiting of pathogenic fungi, except *Bacillus* sp HI that could not inhibit *Foc*. *B. cereus* P14, *B.cereus* Se07, *Bacillus* sp SJI and *S.marcescens* had the ability to inhibit of all three pathogenic fungi (*C. capsici, C. gloeosporiodes* and *Foc*). All endophytic bacteria were able to inhibit *Xaa* but were not able to inhibit *R. solanacearum*. Physiological characteristics of potential endophytic bacteria as biocontrol agents and growth promotor ware the production of

salicylic acid, siderophot, IAA, lipase enzymes, protease enzymes and phosphate solubilization.

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REFERENCES

1. BAKKER, P.A.H.M., PIERTERSE, C.M.J., VAN LOON, L.C., **Induced systemic resistance by Fluorescent *Pseudomonas spp*.**, Phytopathology, vol. 97, 2007, pp. 239-243
2. BERG, G., HALLMANN, J., **Control of plant pathogenic fungi with bacterial endophytes**, In: Microbial root endophytes. Schulz B, Boyle C, Sieber TN, eds. Springer, Berlin, 2006, pp. 53–67.
3. COMPANT, S., DUFFY, B., NOWAK, J., CLÉMENT, C., BARKA, E. A., **Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects**, Appl. Environ. Microb., vol. 71, 2005, pp. 4951–4959.
4. HARDAM, P.R., VAN OVERBEEK, L.S., BERG, G., PIRTTILA, A.M., COMPANT, S., COMPISANO, A., **The hidden world within plants ecological and evolutionary considerations for defining functioning of microbial endophytes**, Microbiol and molecul biology reviews; vol.79, no.3, 2015, pp.293-320.
5. JASIM, B., JIMTHA, J. C., MATHEW, J., RADHAKRISHNAN, E.K., **Plant growth promoting potential of endophytic bacteria isolated from Piper nigrum**, Plant Growth Regul. doi:10.1007/s10725-013- 9802-y, 2013.
6. KLOEPPER, J.W., RYU, C.M., ZHANG, S., **Induced systemic resistance and promotion of plant growth by *Bacillus spp***, Phytophatology, vol. 94, 2004, pp. 1259-1266.
7. KUMAR, A., SINGH, R., YADAV, A., GIRI, D.D., SINGH, P.K., PANDEY, K.D., **Isolation and characterization of endophytes of Curcuma longa. L**, Biotech, vol. 6, 2016, pp. 60-68.
8. LUGTENBERG, B., KAMILOVA, F., **Plant-growth-promoting Rhizobacteria**, Annu Rev Microbiol., vol. 63, 2009, pp. 541–56.
9. MACAGNAN, D., ROMEIRO, R.S., ALAN, W., POMELLA DE SOUZA, J.T., **Production of lytic enzymes and siderophores, and inhibition of germination of basidiospores of *Moniliophthora* (ex *Crinipellis*) *perniciosa* by phylloplane actinomycetes**, Biol Control, vol. 47, 2008, pp.309-314. http://dx.doi.org/10.1016/j.biocontrol.2008.08.016
10. MALFANOVA, N., KAMILOVA, F., VALIDOV, S., CHEBOTAR, V., LUGTENBERG, B., **L-arabinose important for the endophytic lifestyle of *Pseudomonas* spp.?**, Arch Microbiol., vol. 195, 2013, pp. 9-17.
11. MELNICK, R.L., ZIDACK, N.K., BAILEY, B.A., MAXIMOVA, S.N., GUILTINAN, M., BACKMAN, P.A., **Bacterial endophytes: Bacillus spp. from annual crops as potential biological control agents of black pod rot of cacao**, Biological control, vol. 46, 2008, pp. 46–56.
12. NASRUN, **Studi pengendalian hayati penyakit layu bakteri (*Ralfsonia solanacearum*) Nilam dengan *Pseudomonas fluorescens***, Disertasi, Pasca sarjana Universitas Gajah Mada, Yogyakarta, 2005.
13. PEREIRA, P., IBANEZ, F., ROSENBLUETH, M., ETCHEVERRY, M., MARTINEZ-ROMERO, M., **Analysis of the bacterial diversity associated with the roots of maize (*Zea mays* L.) through culture-dependent and culture-independent methods**, ISRN Ecology, doi:10.5402/2011/938546, 2011.
14. RAHMAN, A., SITEPU, I.R., TANG, S.Y., HASHIDOKO, Y., **Salkowski's reagent test as a primary screening index for functionalities of Rhizobacteria isolated from wild dipterocarp saplings growing naturally on medium-strongly acidic tropical peat soil**, Biosci Biotechnol Biochem, vol. 74, 2010, pp.2202–2208.
15. RESTI, Z., HABAZAR, T., PUTRA, D.P., NASRUN, **Skrining dan identifikasi isolat bakteri endofit untuk mengendalikan penyakit hawar daun bakteri pada bawang merah**, J.HPT Tropika, vol. 13, no.2, 2013, pp. 167 –178.

16. RESTI, Z., **Karakterisasi Respon Fisiologis Tanaman Bawang Merah yang diintroduksi dengan bakteri endofit indigenus terhadap penyakit hawar daun bakteri (*Xanthomonas axonopodis pv. allii*)**, Disertasi, Program Pascasarjana Universitas Andalas, 2016.
17. SIERRA, G. A., **A simple method for the detection of lypolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substracts**, Antonine van Leeuwenhoeck, vol. 28, 1957, pp. 15-22.
18. WANG, Y., ZENG, Q., ZHANG, Z., **Antagonistic bioactivity of an endophytic bacterium H-6**, African Journal of Biotechnology, vol. 9, no. 37, 2010, pp. 6140-6145.
19. XU, D., XIA, X., XU, N., AN, C., **Isolation and identification of novel endophytic bacterial strain with antifungal activity from wild blueberry *Vaccinium uliginosum***, Ann Microb., vol. 57, no.4, 2007, pp. 673-676.
20. ZINNIEL, D.K., PAT LAMBRECHT, B., HARRIS, Z., FENG, D., KUCZMARSKI, P., HIGLEY, C.A., ISHIMARU, A., ARUNAKUMARI, R.G., BARLETTA, VIDAVER, A.K., **Isolation dan Characterization of Endophytic Colonizing Bacteria From Agronomic Crops and Prairie Plants**, Applied and Enviromental Mycrobiology, 2002, pp. 2198-2208.
21. ZIVKOVIC, V., STOJANOVIC, Z., IVANOVIC, **Screening of antagonistic activity of microorganisms against c*olletotrichum acutatum* and C*olletotrichum gloeosporioides***, Arch. Biol. Sci., vol. 62, no.3, 2010, pp. 611-623.